

ORIGINAL ARTICLE

Evaluation of the Effect of Different Antiseptic Solutions on Aerobic Bacterial Wound Infection

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ABSTRACT

Key words:

Wound infection,
Hypochlorous acid,
antiseptics

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Background: Wound infection represents a major concern in healthcare system. Many bacterial pathogens can cause this type of infection. Alternative non-antibiotic inhibitors of these pathogens have become a major research concern. Some antiseptic agents, alone or in combination with antibiotics, can be highly effective against the causative organisms. Hypochlorous acid (HOCl) displays bactericidal effects that can be employed *in vivo* on surgical wounds to minimize certain bacterial infections. **Objective:** To investigate the antibacterial effect of HOCl on post-operative wound infection in comparison to common antiseptics e.g. Povidone iodine and alcohol. **Methodology:** This study was carried out in Medical Microbiology & Immunology Department, Faculty of Medicine, Tanta University on fifty patients admitted to Surgical ICU, Tanta University hospital, during the period of research (June to November 2018). Seven swab samples were collected from the wound discharge of each patient. The first swab was directly put into the swab tube. Other 6 swabs were soaked into tubes containing 5 ml of either HOCl solution, 75% alcohol, and Povidone iodine solution and left for one and five minutes respectively. After which, they were subjected to bacterial culturing. **Results:** Among the 50 wound samples, positive cultures were obtained from 27 (54%) directly cultured samples. After soaking for one minute, HOCl soaked swabs revealed bacteria in 20 (40%) patients, those in Povidone Iodine revealed bacteria in 19 (38%) patients, 75% alcohol revealed bacteria in 6 (12%) patients. The bacterial culture results was significantly lower when the swabs were soaked in alcohol (P value > 0.001). After soaking for 5 minutes, swabs in HOCl revealed bacteria in 18 (36%) patients. Swabs soaked in Povidone Iodine revealed bacteria in 18 (36%) patients while swabs in 75% alcohol revealed bacteria in 2 (4%) patients. The bacterial culture rate was significantly lower when the swabs were placed in alcohol. The culture result was lower when swabs were soaked in the 3 solutions for 5 minutes than for one minute, although this difference was non-significant. **Conclusion:** Hypochlorous acid has reasonable antibacterial effect on bacteria isolated from wound discharge. This effect is progressive and time dependent. It should be used for a reasonable period to observe more potent antibacterial activity.

INTRODUCTION

Wound infections are compound multi-factorial processes where bacterial pathogens such as *Pseudomonas aeruginosa* or *Staphylococcus aureus* represent the most important factor targeted during patient treatment.¹

Many studies are concerned with developing alternative non-antibiotic inhibitors of these pathogens. Researchers suggest that some antiseptic agents, alone or in combination with antibiotics, can be highly effective against multi-drug resistant organisms (MDROs).²

Hypochlorous acid (HOCl) is a weak acid which is formed when chlorine disbands in water.³ It can also be produced by the electrolysis of a weak sodium chloride solution.⁴

HOCl is frequently used, in its high concentrations, as a bleaching agent and to disinfect tap or swimming pool water because of its potent sterilizing ability.^{5,6} *In vivo*, our immune cells produce HOCl as an immune mechanism fighting infections.⁷ It is produced by the action of H₂O₂ and neutrophil-derived myeloperoxidase and possesses a potent antibacterial activity as an oxidizing agent.^{8,9} Recently, HOCl solution displayed bactericidal effects when employed as a nasal irrigant to treat pediatric chronic sinusitis.^{10,11}

Some disadvantages of using HOCl have been facing researchers as it was found to be highly corrosive and gradually losing its bactericidal effect with long-term preservation, therefore conditions of long-term storage and safety measures must be applied. Where the safety standard concentration within the World Health

Organization for in vivo use or drinking water is 5 mg/L.¹²

HOCl was reported to have no teratogenic or carcinogenic properties.⁵ Moreover, its solution does not display any cytotoxic or gene-mutating effects.¹³ So the concentration of HOCl used in our study is considered safe for the human body.¹⁴ In the present work, our aim was to investigate the antibacterial effect of HOCl on post-operative wounds' discharge in comparison to common antiseptics e.g. Povidone iodine and alcohol.

METHODOLOGY

This study was approved by the Ethics Committee, Faculty of Medicine, Tanta University. Written consent was obtained from each patient included in this study.

Subjects:

A total of 350 wound samples were collected from 50 patients with post-operative wound infection admitted to Surgical ICU, Tanta University Hospital, between June and November 2018. Infected surgical wounds included in the study were clinically suspected by having at least one of the following signs or symptoms 2 days after surgery: Redness, hotness, swelling, purulent discharge or delayed wound healing. Patients receiving antibiotic therapy during the preceding 7 days were excluded from the study.

Specimen collection and processing:

- All samples were collected with sterile swabs in sterile swab tubes. Seven swab samples were collected from wound discharge of each patient. The first swab was directly put into the swab tube. Then 3 swabs were soaked into 3 tubes containing 5ml of either HOCl solution, 75% alcohol, or Povidone iodine solution and soaked in these solutions for one minute. After which, all the 3 soaked swabs were transferred to sterile swab tubes.
- Hypochlorous acid was prepared in a concentration of (sterile NaCl 0.9% to HOCl 50.5% at ratio 8:2)¹⁴ and the Povidone Iodine solution with a concentration of 10 % was an aliquot from a bottle of Betadine® Antiseptic solution (Nile Pharmaceuticals, Egypt licensed by Mundipharma AG, Switzerland).
- In the same manner, the last 3 swabs were soaked in the same three solutions with the same amount and concentrations for five minutes. Then transferred to sterile swab tubes. All the 7 swabs were transported as soon as possible to the Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University to be subjected to bacterial culturing and identification.

Bacterial Culture:

In the Microbiology laboratory, specimens from the swab tubes were inoculated on blood and MacConkey agar plates, and the plates were incubated in 5%

CO₂ incubator at 37°C for 24-48 hours. All isolates were routinely identified for aerobic bacteria.

Statistical Analysis:

Culture results were compared using Pearson's Chi-square test. It was considered statistically significant when P value < 0.001. SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used to perform all analyses.

RESULTS

Fifty post-operative wound samples were included in this study, comprising 23 females and 27 males samples. The mean age was ± 45.4 years with a range from 35 to 58 years. 7 wound swabs were taken from each patient.

Among the 50 wound samples that were placed directly into swab tubes for direct culture, bacterial isolates were obtained from 27 (54%) patients including 33 different isolates (due to the presence of mixed infection in some cases).

- *Culturing results after soaking swabs for one minute:*

When the swabs were soaked in HOCl solution for one minute, positive cultures were obtained from 20 (40%) patients with 20 different isolates. When the swabs were soaked in Povidone Iodine solution for one minute, positive cultures were obtained from 19 (38%) patients with 19 different isolates. When the swabs were soaked in 75% alcohol for one minute, bacterial isolates were obtained from 6 (12%) patients with 6 different isolates. The bacterial culture rate was significantly lower when the swabs were placed in alcohol (P value < 0.001), but the culture result was not significantly different among the other two groups.

- *Culturing results after soaking swabs for 5 minutes:*

When the swabs were soaked in HOCl solution for 5 minutes, bacterial isolates were positive from 18 (36%) patients with 18 different isolates. When the swabs were soaked in Povidone Iodine solution for 5 minutes, positive cultures were obtained from 18 (36%) patients with 18 different isolates. When the swabs were soaked in 75% alcohol for 5 minutes, bacteria were obtained from 2 (4%) patients with 2 different isolates. The bacterial culture rate was significantly lower when the swabs were placed in alcohol (P value < 0.001), but the culture result was not significantly different among the other two groups.

- *Comparison between culture results after soaking for one and 5 minutes:*

When the culture results of samples which were soaked into the 3 different solutions for one minute were compared with those of samples that were soaked into the same solutions for 5 minutes, the culture result was lower when swabs were soaked in the 3 solutions for 5 minutes than when they were soaked for one minute, although this difference was non-significant (P value > 0.001). The results are shown in table (1).

Table 1: Bacteriology of the four studied groups before and after soaking for one and five minutes

Isolated bacteria	Swab without soaking	HOCl		Povidone Iodine		Alcohol	
		1 min	5 min	1 min	5 min	1 min	5 min
Gram-positive (15)							
<i>Coagulase-negative staphylococci</i>	2	1	1	1	1	--	--
<i>Staphylococcus aureus(MSSA)</i>	5	4	3	3	3	2	--
<i>Staphylococcus aureus(MRSA)</i>	2	2	2	2	2	1	1
<i>Enterococci</i>	2	1	1	1	1	--	--
<i>Streptococcus pyogenes</i>	1	--	--	--	--	--	--
Gram-negative (33)							
<i>Pseudomonas aeruginosa</i>	8	5	4	3	3	1	--
<i>Klebsiella pneumonia</i>	6	4	4	4	4	1	1
<i>Acinetobacter baumannii</i>	2	1	1	2	2	1	--
<i>Proteus mirabilis</i>	1	1	1	1	1	--	--
<i>Escherichia coli</i>	2	1	1	1	1	--	--
<i>Stenotrophomonas maltophilia</i>	1	--	--	--	--	--	--
<i>Enterobacter aerogenes</i>	1	--	--	1	1	--	--
Total aerobic and facultative bacteria	33	20	18	19	18	6	2

DISCUSSION

Wound infections surge the chance of patients' morbidity and mortality and cause a substantial load on health care systems. Wounds can be soiled from the neighboring skin, endogenous or even exogenous sources. There is a widespread application of using topical antiseptics in chronic wounds nowadays. These agents are effective in inhibition of microbial colonization, but in extra-concentrations, they can be cytotoxic or interfere with wound healing.

Many studies showed that some antiseptic solutions have significant anti-microbial action against several organisms, their activities varied according to the type of organism.¹⁵⁻¹⁷

In our study, we investigated the antibacterial activity of hypochlorous acid (HOCl) on pus from post-operative wounds in comparison with Povidine Iodine and alcohol 75%. Our results revealed that the three solutions showed antibacterial effect as the positive culture rate decreased with the usage of the three solutions. Significant lower results were obtained when the swabs were soaked in alcohol for either 1 or 5 minutes, while no significant difference was recorded when HOCl or Povidone iodine were tried.

The results of the current study are concomitant with the previous finding by Serhan et al,¹⁵ who demonstrated that stabilized HOCl solution had strong and fast bactericidal effects on common microorganisms causing wound infections and also had anti-biofilm effects.

Moreover, Wang et al¹⁸ revealed that HOCl had antibacterial activity against certain bacterial species such as *S. aureus*, *P.aeruginosa*, and *C. albicans*. It also

has been reported by Kim et al,³ that a low concentration of HOCl solution established not only powerful antibacterial effects but also antifungal ones.

The results reported by Ragab et al,¹⁴ in a clinical trial, which compared the effectiveness of Hypochlorous Acid versus Hydrogen Peroxide followed by Povidine Iodine as a wound care agent in treating infected diabetic foot ulcers support the previous studies that, HOCl is a greatly effective antimicrobial agent against many bacterial species such as *Proteus*, *Klebsiella*, *Pseudomonas*, and *MRSA* compared with H₂O₂ and Povidone iodine in treating infected diabetic foot ulcers, and they proved it by the rapid formation of healthy granulation tissue without further necrosis and quantitative bacterial result.

On the other hand, Wu et al,¹¹ found that there is no good antibacterial effect after soaking specimens into antiseptic solutions in general. However, this could be explained by the different methodology they applied. As their samples were taken from the discharge of chronic sinusitis and the samples were placed in contact with the antiseptic solutions before inoculation for different durations than those applied in our study.

In our study, the bactericidal effect of HOCl, Povidone iodine and alcohol increased after 5 minutes contact, although non-significantly, but this can lead us to the possibility of using HOCl as wound antiseptic, but with putting in mind that the contact duration should be long enough to allow its antibacterial effect to be evident.

Erilmaz et al,⁷ approved that HOCl is a strong antimicrobial agent. Their results focused on its advantages as the non-toxicity in living tissues and environmental safety, but they also clinched that it has

restricted applications due to its diminishing antimicrobial efficacy in the presence of organic material along with low storage stability suggesting that the determination of HOCl with various analytical methods are required. Nevertheless, the antibacterial effect of HOCl was not assessed in their study because bacterial cultures were not conducted.

CONCLUSION

Hypochlorous acid seems to be an active reasonably strong antibacterial solution. It has many advantages as being inexpensive and easy to prepare. This study results showed that HOCl solution exhibits progressive anti-bacterial effect on bacteria in the wound discharge within a short time. We hypothesize that irrigation of the wound with an antibacterial fluid such as HOCl for a reasonable period may be needed to observe more potent antibacterial activity. Thus, further studies are needed to determine whether exposing wound to HOCl or Povidone iodine solution for duration of longer than 5 minutes would exert a greater antibacterial effect. Also further experimental studies are needed to confirm absence of any cytotoxic effects of hypochlorous solution when used in-vivo.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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